

Segm fold from 1/2

1 GAAGTGCAGCGGGGTGGATTTCCTGGAATTGCCTTAGTAGTAGTACCACCCAAGGCACCTG
61 CTTAGGTACCAGTCTGCTTAGTGGAGAGTCCCTCTGGCTTTATCATTAAAGGTTTTGGGC
121 GGAAAGACGTAGTTGAATATTTGCTTCAGAATGGTGCAAATGTCCAAGCACGTGATGATG
181 GGGGCCCTTATTCCTCTTCATAATGCATGCTCTTTTGGTCATGCTGAAGTAGTCAATCTCC
241 TTTTGCACATGGTGCAGACCCCAATGCTCGAGATAATTGGAATTATACTCCTCTCCATG
301 AAGCTGCAATTAAAGGAAAGATTGATGTTTTGCATTGTGTTGCTATTTTGCAGTGTCTGTTA
361 CAGCATGGAGCTGAGCCAACCATCCTAAATACAGATGGAAGGACAGCATTGGATTTAGCA
421 GATCCATCTGCCAAAGCAGTGCTTACTGGTGAATATAAGAAAGATGAACTCTTAGAAAGT
481 GCCAGGAGTGGCAATGAAGAAAAAATGATGGCTCTACTCACACCATTAATGTCAACTGC
541 CACGCAAGTGATGGCAGAAAGTCAACTCCATTACATTTGGCAGCAGGATATAACAGAGTA
601 AAGATTGTACAGCTGTTACTGCAACATGGAGCTGATGTCCATGCTAAAGATAAAGGTGAT
661 CTGGTACCATTACACAATGCCGTGTTCTTATGGTCATTATGAAGTAACTGAACTTTTGGTC
721 AAGCATGGTGCCTGTGTAAATGCATGGACTTTGTGGCAATTCACCTCTTCTATGAGGCA
781 GCTTCTAAGAACAGGGTTGAAGTATGTTCTCTTCTTAAGTTATGGTGCAGACCCAACA
841 CTGCTCAATTGTCACAATAAAAGTGCTATAGACTTGGCTCCCACACCCACAGTTAAAGAA
901 AGATTAGCATATGAATTTAAAGGCCACTCGTTGCTGCAAGCTGCACGAGAAGCTGATGTT
961 ACTCGAATCAAAAAACATCTCTCTCTGGAATGGTGAATTTCAAGCATCCTCAAACACAT
1021 GAAACAGCATTGCATTGTGCTGCTGCATCTCCATATCCCAAAAGAAAGCAAATATGTGAA
1081 CTGTTGCTAAGAAAAGGAGCAAACATCAATGAAAAGACTAAAGAATTCTTGACTCCTCTG
1141 CACGTGGCATCTGAGAAAGCTCATAATGATGTTGTTGAAGTAGTGGTGAACATGAAGCA
1201 AAGGTTAATGCTCTGGATAATCTTGGTCAGACTTCTCTACACAGAGCTGCATATTGTGGT
1261 CATCTACAAACCTGCCGCCCTACTCCTGAGCTATGGGTGTGATCCTAACATTATATCCCTT
1321 CAGGGCTTTACTGCTTTACAGATGGGAAATGAAAATGTACAGCAACTCCTCCAAGAGGGT
1381 ATCTCATTAGGTAATTCAGAGGCAGACAGACAATTGCTGGAAGCTGCAAAGGCTGGAGAT
1441 GTCGAAACTGTAAAAAACTGTGTACTGTTTCAGAGTGTCAACTGCAGAGACATTGAAGGG
1501 CGTCAGTCTACACCATTTCATTTTGCAGCTGGGTATAACAGAGTGTCCGTGGTGAATAT
1561 CTGCTACAGCATGGAGCTGATGTGCATGCTAAAGATAAAGGAGGCCTTGTACCTTTGCAC
1621 AATGCATGTTCTTATGGACATTATGAAGTTGCAGAACTTCTTGTTAAACATGGAGCAGTA
1681 GTTAATGTAGCTGATTTATGGAAATTTACACCTTTACATGAAGCAGCAGCAAAAGGAAAA
1741 TAGAAATTTGCAAACTTCTGCTCCAGCATGGTGCGAGACCCCTACCAAAAAAAGCAGGGAT
1801 GGAAATACTCCTTTGGATCTTGTTTAAAGATGGAGATACAGATATTACATTATCTGCTTAGG
1861 GGAGATGCAGCTTTGCTAGATGCTGCCAAGAAGGGTTGTTTAGCCAGATGAAGAAGTTG
1921 TCTTCTCCTGATAATGTAATTTGCCGCGATACCCAAGGCAGACATTCAACACCTTTACAT

1981 TTAGCAGCTGGTTATAATAATTTAGAAGTTGCAGAGTATTTGTTACAACACGGAGCTGAT
 2041 GTGAATGCCCCAAGACAAAGGAGGACTTATTCCTTTACATAATGCAGCATCTTACGGGCAT
 2101 GTAGATGTAGCAGCTCTACTAATAAAGTATAATGCATGTGTCAATGCCACGGACAAATGG
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 2281 GTTTCAGCGGATGATGTGTCAGCGCTCTTCTGACAGCAGCCATGCCCCCATCTGCTCTGCCC
 2341 TCTTGTTACAAGCCTCAAGTGCTCAATGGTGTGAGAAGCCCAGGAGCCACTGCAGATGCT
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 2581 CTTGGACTTGAGCACCTAATGGATATATTTGAGAGAGAACAGATCACTTTGGATGTATTA
 2641 GTTGAGATGGGGCACAAGGAGCTGAAGGAGATTGGAATCAATGCTTATGGACATAGGCAC
 2701 AAACATAATTAAGGAGTCGAGAGACTTATCTCCGACAACAAGGTCTTAACCCATATTTA
 2761 ACTTTGAACACCTCTGGTAGTGGAACAATTCTTATAGATCTGTCTCCTGATGATAAAGAG
 2821 TTTTCAGTCTGTGGAGGAAGAGATGCAAAGTACAGTTCGAGAGCACAGAGATGGAGGTCAT
 2881 GCAGGTGGAATCTTCAACAGATACAATATTCTCAAGATTGAGAAGGTTTGTAACAAGAAA
 2941 CTATGGGAAAGATACACTCACCGGAGAAAAGAAGTTTCTGAAGAAAACCACAACCATGCC
 3001 AATGAACGAATGCTATTTTCATGGGTCTCCTTTTGTGAATGCAATTATCCACAAAGGCTTT
 3061 GATGAAAGGCATGCGTACATAGGTGGTATGTTTGGAGCTGGCATTATTTTGTGCTGAAAAC
 3121 TCTTCCAAAAGCAATCAATATGTATATGGAATTGGAGGAGGTACTGGGTGTCCAGTTCAC
 3181 AAAGACAGATCTTGTTACATTTGCCACAGGCAGCTGCTCTTTTGGCCGGTAACCTTGGGA
 3241 AAGTCTTTCTGTCAGTTCAGTGCAATGAAAATGGCACATTCTCCTCCAGGTCATCACTCA
 3301 GTCAGTGGTAGGCCCAGTGTAATGGCCTAGCATTAGCTGAATATGTTATTTACAGAGGA
 3361 GAACAGGCTTATCCTGAGTATTTAATTACTTACCAGATTATGAGGCCTGAAGGTATGGTC
 3421 GATGGATAAATAGTTATTTTAAAGAACTAATCCACTGAACCTAAAATCATCAAAGCAGC
 3481 AGTGGCCTCTACGTTTTACTCCTTTGCTGAAAAA

ref|NP_003738.1|PTNKS| TANKYRASE >gi|3929219 (AF082556) TRF1-interacting
 ankyrin-related

ADP-ribose polymerase [Homo sapiens] Length = 1327
 Score = 1640 bits (4199), Expect = 0.0

Identities = 790/1023 (77%), Positives = 871/1023 (84%), Gaps = 11/1023 (1%)
 Query: 35 VLLQHGAEPITLNTDGRALDLADPSAKAVLTGEYKKDELLESARSGNEEKMMALLTPLN 94
 VLLQHGA+P I NTDG++ALDLADPSAKAVLTGEYKKDELLE+ARSGNEEK+MALLTPLN
 Sbjct: 300 VLLQHGAADPNIRNTDGKSALDLADPSAKAVLTGEYKKDELLEAARSGNEEKLMMALLTPLN 359
 Query: 95 VNCHASDGRKSTPLHLAAGYNRVKIVQLLLQHGADVHAKDKGDLVPLHNACSYGHYEVT 154
 VNCHASDGRKSTPLHLAAGYNRV+IVQLLLQHGADVHAKDKG LVPLHNACSYGHYEVT
 Sbjct: 360 VNCHASDGRKSTPLHLAAGYNRVIVQLLLQHGADVHAKDKGGLVPLHNACSYGHYEVT 419
 Query: 155 LLVKHGACVNAMDLWQFTPLHEAASKNRVEVC SLLLSYGADPTLLNCHNKSALDLAPTPQ 214
 LL+KHGACVNAMDLWQFTPLHEAASKNRVEVC SLLLS+GADPTL+NCH KSA+D+APTP+
 Sbjct: 420 LLLKHGACVNAMDLWQFTPLHEAASKNRVEVC SLLLSHGADPTLVNCHGKSADVMAPTPE 479
 Query: 215 LKERLAYEFKGHSLQAAREADVTRIKKHLSELMVNFKHPQTHETALHCAAASPYPKRKQ 274
 L+ERL YEFKGHSLQAAREAD+ ++KK L+LE++NFK PQ+HETALHCA AS +PKRKQ
 Sbjct: 480 LRERLTYEFKGHSLQAAREADLAKVKKTLALEIINFKQPQSHETALHCAVASLHPKRKQ 539
 Query: 275 ICELLLRKGANINEKTKEFLTPLHVASXXXXXXXXXXXXXXXXXXXXLDNLGQTS LHRAA 334
 + ELLLRKGAN+NEK K+F+TPLHVA+ LD LGQT+LHRAA
 Sbjct: 540 VTELLLRKGANVNEKNKDFMTPLHVAAERAHNDVMEVLHKKHGAKMNALDTLGQTALHRAA 599
 Query: 335 YCGLHQTCLLLSYGCDPNIIISLQGF TALQMGNE NVQQLLQEGISLGNSEADRQLLEAAK 394
 GHLQTCLLLSYG DP+IISLQGF TA QMGNE VQQ+L E + S+ D +LLEA+K
 Sbjct: 600 LAGHLQTCLLLSYGSDPSIIISLQGF TAAQMGNEAVQQILSESTPIRTSDVDYRLLEASK 659

Query: 395 AGDVETVKKLCTVQSVNCRDIEGRQSTPLHFAAGYNRVSVVEYLLQHGADVHAKDKGGLV 454
 AGD+ETVK+LC+ Q+VNCRD+EGR STPLHFAAGYNRVSVVEYLL HGADVHAKDKGGLV
 Sbjct: 660 AGDLETVKQLCSSQNVNCRDLEGRHSTPLHFAAGYNRVSVVEYLLHHGADVHAKDKGGLV 719

Query: 455 PLHNACSYGHYEVAELLVKHGAVNVADLWKFTPLHEAAAKGKYEICKLLLQHGDPTKK 514
 PLHNACSYGHYEVAELLV+HGA VNVADLWKFTPLHEAAAKGKYEICKLLL+HGADPTKK
 Sbjct: 720 PLHNACSYGHYEVAELLVRHGASVNVADLWKFTPLHEAAAKGKYEICKLLLKHGADPTKK 779

Query: 515 NRDGNTPLDLVKDGDTDIHYXXXXXXXXXXXXXXXXXXXXRVKKLSSPDNVNCRDTQGRHST 574
 NRDGNTPLDLVK+GDTDI RV+KL +P+N+NCRDTQGR+ST
 Sbjct: 780 NRDGNTPLDLVKEGDTDIQDLLKGDAALLDAAKKGCLARVQKLCTPENINCRDTQGRNST 839

Query: 575 PLHLAAGYNNLEVAEYLLQHGADVNAQDKGGLIPLHNAASYGHVDVAALLIKYNACVNAT 634
 PLHLAAGYNNLEVAEYLL+HGADVNAQDKGGLIPLHNAASYGHVD+AALLIKYN CVNAT
 Sbjct: 840 PLHLAAGYNNLEVAEYLLLEHGADVNAQDKGGLIPLHNAASYGHVDIAALLIKYNTCVNAT 899

Query: 635 DKWAFTPLHEAAQKGRTQLCALLAHGADPTLKNQEGQTPLDLVSADDVSALLTAAMPPS 694
 DKWAFTPLHEAAQKGRTQLCALLAHGADPT+KNQEGQTPLDL +ADD+ ALL AMPP
 Sbjct: 900 DKWAFTPLHEAAQKGRTQLCALLAHGADPTMKNQEGQTPLDLATADDIRALLIDAMPPE 959

Query: 695 ALPSCYKPQ---VLNGVRSPGATXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXX 751
 ALP+C+KPQ V + SP +T
 Sbjct: 960 ALPTCFKPQATVVSASLISPAST-----PSCLSAASSIDNLTGPLAELAVGGASNAG 1011

Query: 752 XXXXXXXXXXXKKEVPGVDFSITQFVRNLGLEHLMDIFEREQITLDVLVEMGHKELKEIGIN 811
 + EV G+D +I+QF+++LGLEHL DIFE EQITLDVL +MGH+ELKEIGIN
 Sbjct: 1012 DGAAGTERKEGEVAGLDMNISQFLKSLGLEHLRDFEFETEQITLDVLADMGHEELKEIGIN 1071

Query: 812 AYGHRHKLIGVERLISGQQGLNPYLTNTSGSGTILIDLSPDDKEFQSVEEEMQSTVRE 871
 AYGHRHKLIGVERL+ GQQG NPYLT + GTIL+DL+P+DKE+QSVEEEMQST+RE
 Sbjct: 1072 AYGHRHKLIGVERLLGGQQGTNPYLTTFHCVNQGTILLDLAPEDKEYQSVEEEMQSTIRE 1131

Query: 872 HRDGGHAGGIFNRYNLIKIQVCNKKLWERYTHRRKEVSEENHNHANERMLFHGSPFVNA 931
 HRDGG+AGGIFNRYN+++IQKV NKKL ER+ HR+KEVSEENHNH NERMLFHGSPF+NA
 Sbjct: 1132 HRDGGNAGGIFNRYNVIRIQVNVNKKLRERFCHRQKEVSEENHNHNERMLFHGSPFINA 1191

Query: 932 IIHKGFDERHAYIGGMFGAGIYFAENSSKSNQYVYGIGGGTGCPVHKDRSCYICHRQLLF 991
 IIHKGFDERHAYIGGMFGAGIYFAENSSKSNQYVYGIGGGTGCP HKDRSCYICHRQ+LF
 Sbjct: 1192 IIHKGFDERHAYIGGMFGAGIYFAENSSKSNQYVYGIGGGTGCPVHKDRSCYICHRQMLF 1251

Query: 992 CRVTLGKSFLQFSAMKMAHSPPGHHSVTGRPSVNGLALAEYVIYRGEQAYPEYLITYQIM 1051
 CRVTLGKSFLQFS MKMAH+PPGHHSV GRPSVNGLA AEYVIYRGEQAYPEYLITYQIM
 Sbjct: 1252 CRVTLGKSFLQFSTMKMAHAPPGHHSVIGRPSVNGLAYAEYVIYRGEQAYPEYLITYQIM 1311

Query: 1052 RPE 1054
 +PE
 Sbjct: 1312 KPE 1314

 GAAGTGCAGCGGGGTGGATTTCCTGGAATTGCCTTAGTAGTAGTACCACCAAGGCACTG
 CTTAGGTACCACTGCTGCTTAGTGGAGAGTCCCTCTGGCTTTATCATTAAGGTTTTGGG
 CGGAAAGACGTAGTTGAATATTTGCTTCAGAATGGTGCAAATGTCCAAGCACGTGATGAT
 GGGGGCCTTATTCCTCTTCATAATGCATGCTCTTTTGGTCATGCTGAAGTAGTCAATCTC
 CTTTTCGACATGGTGCAGACCCCAATGCTCGAGATAATTGGAATTATACTCCTCTCCAT
 GAAGCTGCAATTAAAGGAAAGATTGATGTTTGCATTTGTGTGCTATTTTGCAGTGCTGT
 TACAGCATGGAGCTGAGCCAACC
 ATCCTAAATACAGATGGAAGGACAGCATTTGGATTTAGCAGATCCATCT

Exhibit B

09/863, 169

w/ Tank northern
blob
→ This slide is for Jaseen

Chk1 two-hybrid screening

Bait: Chk1

is a protein kinase required for cell cycle arrest in response to DNA damage

Hit: a novel protein homology to ATP-dependent RNA helicase
belongs to the DEAD-box RNA helicase family

The fission yeast *cdc28(+)* encodes a member of the DEAD-box family of putative RNA helicases involved in pre-mRNA splicing and cell cycle progression

a new gene encoding a putative DEAD box helicase have been isolated to suppress uncontrolled mitosis by overexpression *cdc25* in fission yeast
(Chk1 and 14-3-3 proteins also show up in this screening)

It is interesting to characterize the interaction of Chk1 and the novel RNA helicase and its role in cell cycle control

Potential targets for further pursuing

p21 hit:	Tankyrase homolog
Traf4 hit:	Cdk liked kinase
hRad9 hit:	PP5
PNCA hits:	a novel helicase a human homolog of SNM1 a novel endo/exo-ribonuclease
Chk1 hit:	an ATP-dependent RNA helicase homolog

Target validation:

- full length cloning
- examine the RNA expression in tumor verse normal tissues
- peptide binding library screening in YTH---->functional assay
- generate dominant-negative mutant

p21 hit: a Tankyrase homolog

Tankyrase (a poly(ADP-ribose) polymerase at human telomeres)

- a protein with homology to ankyrin and to the catalytic domain of ADP-ribose polymerase (PARP)
- is localized to human telomeres
- binds to the telomeric protein TRF1 (telomeric repeat binding factor-1)
- is a positive regulator of telomere length maintenance

From Page No. _____

See p87.

7/9/99 (5)

SS Syn

HB (DT)

HB (RL)

polymer HB (1497)

Smart Otago II (9040495)

0.5% (A)

0.5% (RL)

primer

FCDs

1A

#2 (RL)

1A

H₂O

2.5

2.5

70°C 3' ice

5A

2.5

5A 10

0.1M DTT

2A

dNTP mix

1

RT

1

10A

42°C 15A+

+50A Tricine-SDTA Buffer, 72°C 7' ice.
(10 ng/μl)

#2 (CZPS)

upm NRP

RL

RL

PCR

upm RL

upm RL

HB (DT)

3A

HB (RL)

3A

Cap 30

7/12/99

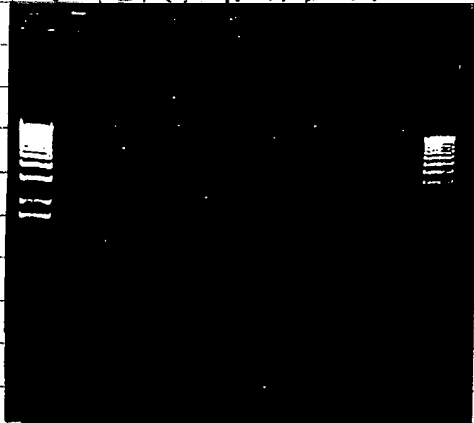
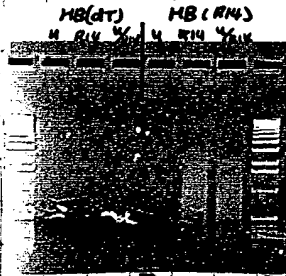
PCR (2D)

primers: NRP RL N/RL

HB (DT) 1P (4/RL) 0.5A

HB (RL) 1P (4/RL) 0.5A

Cap 23



X

#2 Cap 21 (N/RL)

#2 Cap 22 (N/RL)

7/12/99

T/AE 10/10

To Page No. _____

Witnessed & Understood by me,

JH

Date

8/27/99

Invented by

SJB

Recorded by

SJB

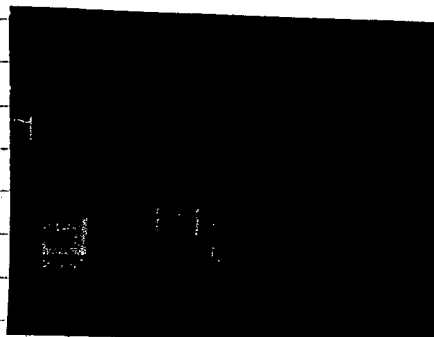
Date

7/9/99

TITLE _____

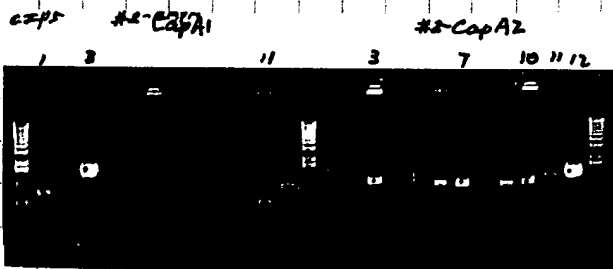
From Page No. _____

Repeat IP but use N/A prime
 PCR primer: NUP RII N/RII
 templates: HB (AT) 2.1
 HB (AT) 2.2
 Cap30. 2.10
 X

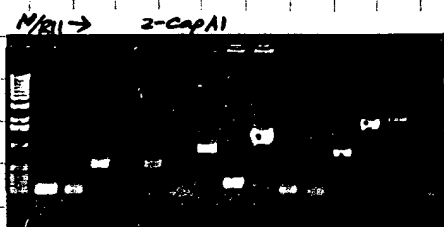


7/13 ② #2-CapA1 & #2-CapA2

✓ PCR insert screening primer: NUP/RII

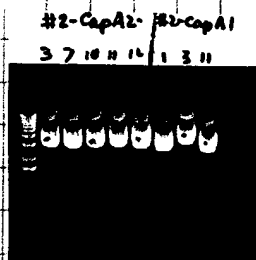


7/19 ① screening more



7/14 ③

mini plasmid prep



#2-CapA2-3 (N/R11)
 -10
 -12

#2-CapA1-3
 -11

300 nM each

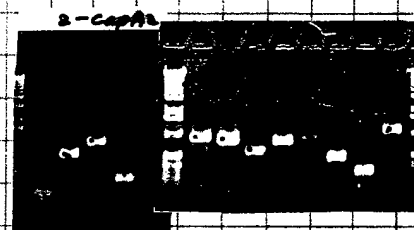
Result
 no sequence

7/14 ③
 to sequence

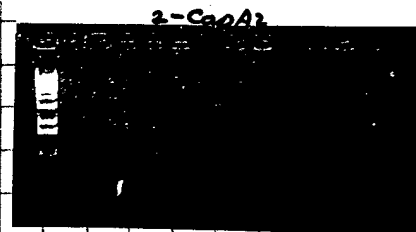
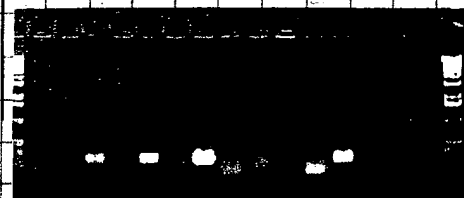
isoform 1

isoform 3

7/12

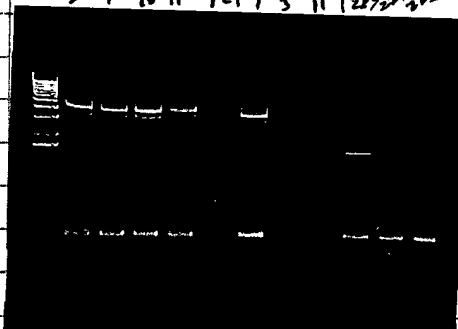


FB/R11 check



#2, per check FB/R11!

#2-CapA2- 3 7 10 11 1 3 11 28/20/205



mini 7/10

To Page No. 137

Witnessed & Understood by me,

JH

Date

8/27/99

Invented by

RS

Recorded by

RS

Date

7/13/99

From Page No. 115

7/13/99 ① 5'-end cloning.

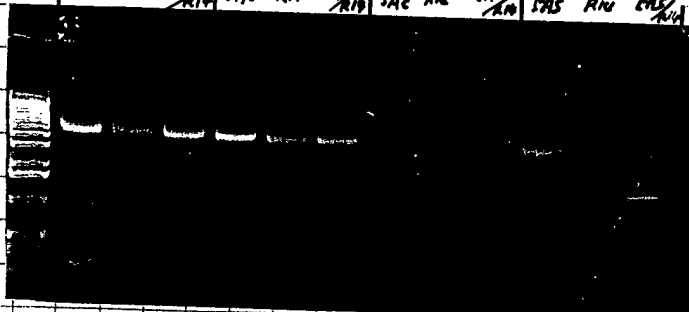
from library

Template:

- | | | | |
|---|--------------|--------|---------|
| ① | HB/plib | primer | SAL/R14 |
| ② | H. Leuk/plib | " | " |
| ③ | 4zap mix | " | SA2/R14 |
| ④ | HB/psport | " | SA5/R14 |

Gap30 (2'40")

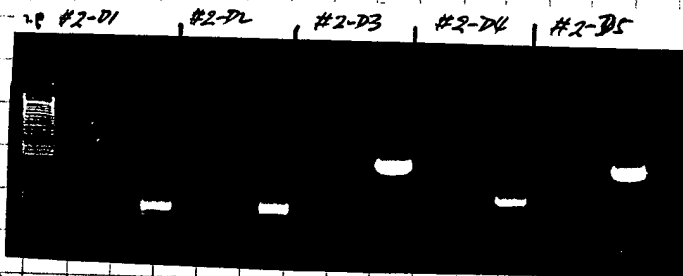
HB/plib	H. Leuk/plib	4zap mix	HB/psport
SAL R14	SAL R14	SA2 R14	SA5 R14



7/13/99 ②

2P
5B/R11 5B/R11
#2-D1
2
3
4
5
Cap23

GP GP GP GP
#2-D1 (SAL/R14) #2-D2 (SAL/R14) #2-D3 (SA2/R14) #2-D4 (SA5/R14)
#2-D2 " " " " " " " "
insert screening 2-D3, 2-D4 only
T/A cloning & 2P

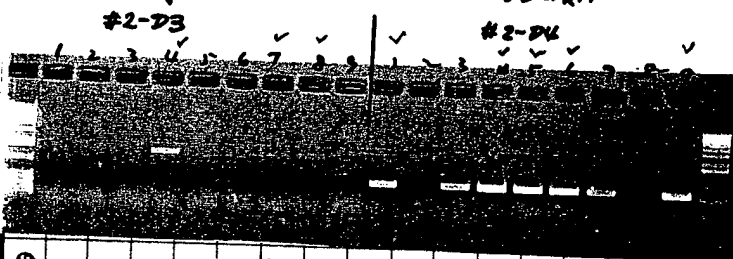


7/14

PCR insert screening, primer 5B2/R11

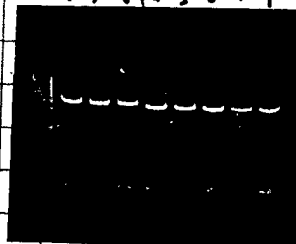
GP GP GP GP GP
#2-E1 (5B2/R11) #2-E2 (5B2/R11) #2-E3 (5B2/R11) #2-E4 (5B2/R11) #2-E5 (5B2/R11)
X X X X X

PCR insert screening, primer 5B2/R11



To sequence 7/14 ①
AT/A cloning
check insert with F13/R11 (11)

2-D3- 2-D4-
4 7 8 4 5 6 1 9

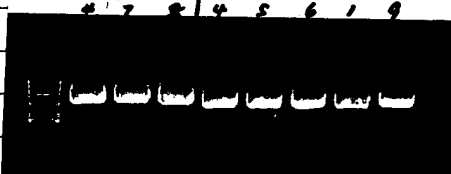


7/15 ③

mini prep

2ul on gel
cath

Cote = 300 u/A



Witnessed & Understood by me,

JH

Date

8/21/99

Invented by

Sy

Recorded by

Sy

Date

7/17/99

To Page No. _____

TITLE

pcmt140 #4.

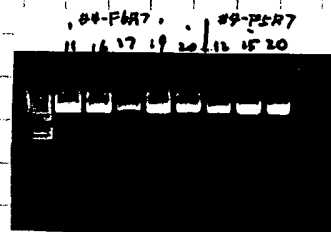
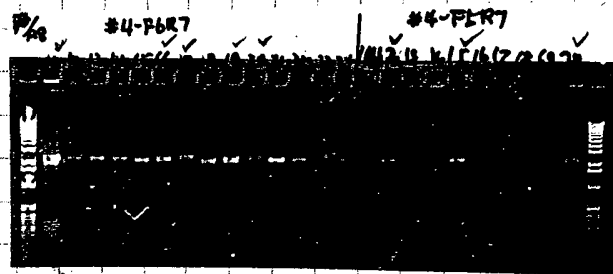
Project No. _____

Book No. _____

127

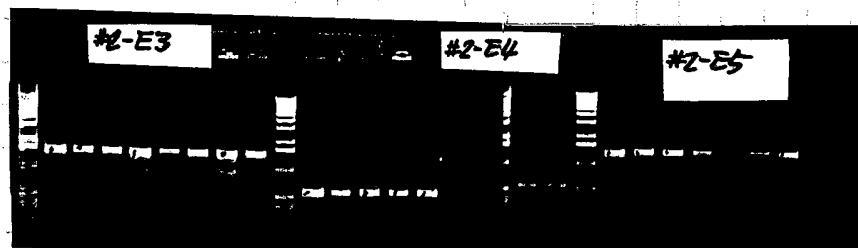
From Page No. 121

7/12/99 ① per insert screening again (last time the amount clones are not enough)
primer use FS/K8



#4-F6R7-11 } 7/13
-19 } to sequence
-20 }
#4-F5R7-18 }

7/15/99 Clones per insert screening



7/20/99 ① 7/13/RH insert check result: all of them have band.

To Page No. _____

Witnessed & Understood by me,

JH

Date

8/1/99

Invented by

SN

Recorded by

SY

Date

7/13/99

From Page No. 124

7/1/99 (2) Compare pH of PCR Buffer.

API R14 API R14

template H₂O (Marathon) 300µl 1. in 25µl PCR
 + 0.5µl 1M HCl + 0.2µl 1M HCl
 + 1.5µl " + 0.5µl "
 + 2.5µl " + 0.8µl "

Cap30
nonuseful

HCl

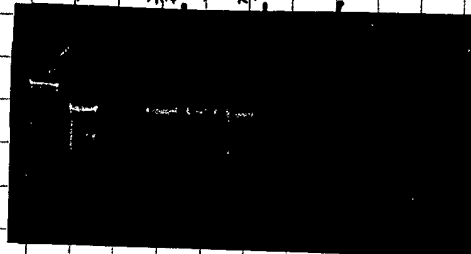
0

0.2µl

0.5µl

0.8µl

API R14 API R14 API R14 API R14

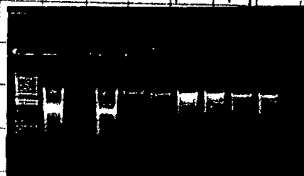


X

Try New buffer & API.

API R14 API R14

API R14 API R14 API R14 API R14 New Buff. Primer 3



X

To Page No. _____

Witnessed & Understood by me,

JH

Date

8/2/99

Invented by

GJ

Recorded by

GJ

Date

7/15/99

7/6/99 (1999) (2-5-) (page date) (change) (sequence)

1. ~~HB~~ { T+B X 6/25, p111 } { Beech kit X } { #2-B4-1 (1999) X }
 { H. leuk/pl V (R14, R11) } { clonech kit V } { -3 }
 { -4 }

2. { HB(R) V 6/30, p115-7 } { #2-C1-6 (1999) }
 { T+B V R14 } { (HB) -7 }
 { H. Mala X } { -12 }
 { H. Liver/pl V } { -18 }
 { #2-C2-1 (1999) V }
 { (HB) -5 }
 { #2-C3-11 (1999) }
 { (H. Liver) -17 }
 { -18 }

3. { HB/pl V 7/2, p126-7 } X HB too small 9/9/99 mini & clones
 { H. leuk/pl V R14 } 15 kb → but didn't pass F13/R11 check X
 { 4 Zap mix V } 4 kb → ∴ no to sequencing
 { H. B/ps V } X too many colonies plate give petk ap to R14
 { #2-D3 (H. leuk) }
 { #2-D4 (4 Zap mix) }
 { #2-D5 (H. B/ps) }

4. ~~HB~~ myself X 7/5, p122, 128 X primer & adaptor problem
 HB R14 Normal Prot different PH but C. New buffer

5. clonech Marathon Ready cDNA, 7/6, p119
 { H. Fetal Brain X }
 { H. Fetal Liver X }
 { H. Leukocyte X }

6. ~~HB~~ 7/9, p14-5. ✓
 { HB primed T ✓ }
 { HB, " R14 X }

#2-CapA1-3 (15kb) V isoform 1
 -11 (0.5kb) V isoform 3
 #2-CapA2-3 (1kb)
 -10
 -12 (1.2kb) V no seq

(Mass F13/R11 check)
 #2-CapA1-29 (1.7kb) V
 -33 (1.2kb) V
 -34 (2.2kb) V
 #2-CapA2-26 (1.1kb) V
 -29 (0.9kb) V

Result: got 2 isoform from Smart RACE. & got isoform from Library method.
 But Marathon did work.
 7/22 finish it.

SCIENTIFIC NOTEBOOK CO.

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TITLE _____

CZPS. #2

From Page No. 125

< min prep. 21 each

1 conc = 200 ng/ μ l



- F13/R11 insert check again.

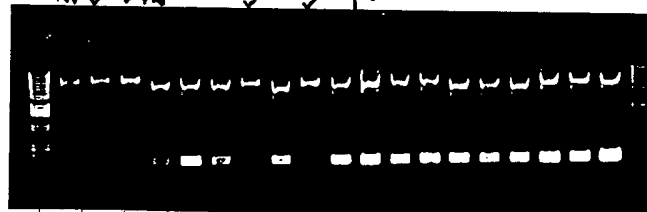
#2-CapA1-29

-33

-34

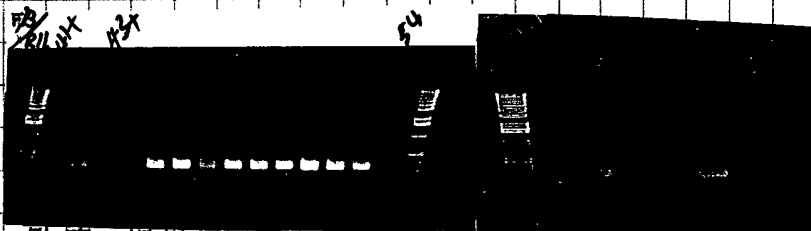
to sequence 7/20 (2)

F13/R11
CZPS
2-CapA1-29
-33
-34
2-CapA1-31
-33
-34
2-CapA1-32
-33
-34
2-CapA1-33
-33
-34
2-CapA1-34
-33
-34
2-CapA1-35
-33
-34
2-CapA1-36
-33
-34
2-CapA1-37
-33
-34
2-CapA1-38
-33
-34
2-CapA1-39
-33
-34
2-CapA1-40
-33
-34
2-CapA1-41
-33
-34
2-CapA1-42
-33
-34
2-CapA1-43
-33
-34
2-CapA1-44
-33
-34
2-CapA1-45
-33
-34
2-CapA1-46
-33
-34
2-CapA1-47
-33
-34
2-CapA1-48
-33
-34
2-CapA1-49
-33
-34
2-CapA1-50
-33
-34
2-CapA1-51
-33
-34
2-CapA1-52
-33
-34
2-CapA1-53
-33
-34
2-CapA1-54
-33
-34
2-CapA1-55
-33
-34
2-CapA1-56
-33
-34
2-CapA1-57
-33
-34
2-CapA1-58
-33
-34
2-CapA1-59
-33
-34
2-CapA1-60
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-34
2-CapA1-61
-33
-34
2-CapA1-62
-33
-34
2-CapA1-63
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-34
2-CapA1-64
-33
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2-CapA1-66
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-34
2-CapA1-67
-33
-34
2-CapA1-68
-33
-34
2-CapA1-69
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2-CapA1-70
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-33
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2-CapA1-72
-33
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2-CapA1-73
-33
-34
2-CapA1-74
-33
-34
2-CapA1-75
-33
-34
2-CapA1-76
-33
-34
2-CapA1-77
-33
-34
2-CapA1-78
-33
-34
2-CapA1-79
-33
-34
2-CapA1-80
-33
-34
2-CapA1-81
-33
-34
2-CapA1-82
-33
-34
2-CapA1-83
-33
-34
2-CapA1-84
-33
-34
2-CapA1-85
-33
-34
2-CapA1-86
-33
-34
2-CapA1-87
-33
-34
2-CapA1-88
-33
-34
2-CapA1-89
-33
-34
2-CapA1-90
-33
-34
2-CapA1-91
-33
-34
2-CapA1-92
-33
-34
2-CapA1-93
-33
-34
2-CapA1-94
-33
-34
2-CapA1-95
-33
-34
2-CapA1-96
-33
-34
2-CapA1-97
-33
-34
2-CapA1-98
-33
-34
2-CapA1-99
-33
-34
2-CapA1-100
-33
-34



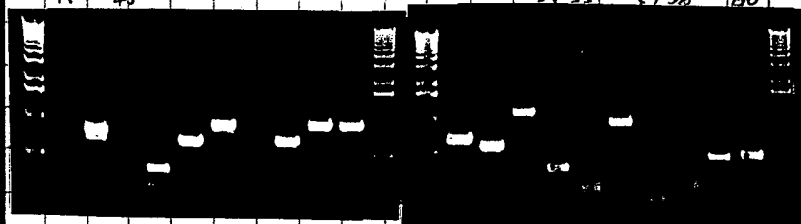
7/21 (3) Pick 20 clones from #2-CapA1

PCR insert screening Nup/R11 & F13/R11



N/R11 PCR

41 43



Restriction A2 PCR assay

#2-CapA1-50 (N/R11)

mini

(See p135)

#2-CapA1-60

CZPS

#2-CapA1-60

to sequence (7/21)

To Page No. _____

Witnessed & Understood by me,

JH

Date

8/17/99

Invented by

SY

Recorded by

SY

Date

7/21/99

RIGEL

4931

FLEHR, HONBACH, TEST
ALBRITTON & HERBERT

1999 JUL 22 AM 9:02

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July 20, 1999

RIGEL, INC.

VIA FEDERAL EXPRESS

Ms. Robin Silva
Flehr, Hobach, Test, Albritton, & Herbert
4 Embarcadero Center, Suite 3400
San Francisco, California 94111-4187

Per RMS - OPEN
AS USPTO

Re: Provisional Patent Applications.

Dear Ms. Silva,

Per Brian Cunningham's request, enclosed with this letter are eight packages of information generated by Dr. Ying Luo in preparation for provisional patent application filings. Each package pertains to a different genetic sequence that Rigel believes may be commercially useful. Each package contains relevant scientific materials, journal references and abstracts of proposed gene functions.

Please file a provisional patent application for each document.

If you have any questions, please call me at 650-624-1106.

Respectfully yours,

Nicole Verona

Nicole A. Verona
Rigel Pharmaceuticals, Inc.

Exhibit E 09/843,149



FLEHR, HOBACH, TEST
ALBRITTON & HERBERT

1999 JUL 23 AM 10:04

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July 22, 1999

RIGEL, INC.

VIA FEDERAL EXPRESS

Ms. Robin Silva
Flehr, Hobach, Test, Albritton, & Herbert
4 Embarcadero Center, Suite 3400
San Francisco, California 94111-4187

*ORIGINAL Diskette in
P-68287*

Re: Provisional Patent Applications.

Dear Ms. Silva,

It was a pleasure to meet you today. I'm sorry that I did not see you leave; I had intended to give you these diskettes before the end of our meeting.

On these diskettes are the documents that we reviewed earlier. The new document that Ying gave to me today will be ready on Monday.

If you have any questions, please call me at 650-624-1106.

Respectfully yours,

Nicole Verona

Nicole A. Verona
Rigel Pharmaceuticals, Inc.

Exhibit # F 09/843,149

DOCKETING/BILLING SYSTEM FILE INFORMATION
(Patent/Design Patent)

Date: July 26, 1999 File No.: A-68292
Client: Rigel Pharmaceuticals Access Code: 4931
Client Attorney: DJB/RMS/DAV
Ref. No.:

New ☒ Update ☐ Close ☐
Parent ☐ Div. ☐ CPA ☐ CIP ☐

Subject Description

Title: TANKYRASEH, A Cell Cycle Protein

Inventors: Ying Luo

Serial No.:
Filing Date:

Patent No.:
Issue Date:

Assignee:

Related Files:

If Foreign file, please provide corresponding U.S. Serial Number
or Patent Registration Number.

Misc. (Include any action items and due dates here!):

Submitted by: Gail Clark Date: July 26, 1999

cc: Accounting
Docketing - Foreign
Docketing - US

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Exhibit AG 09/843, 149

From: Nicole Verona <NVerona@rigel.com>
To: "'dvance@flehr-iplaw.com'" <dvance@sfpo.fhtah.fleh...
Date: 8/30/99 4:01pm
Subject: FW: FW: info

Dear Dolly,

I forwarded your questions to Ying Luo and this is the response I received from him. I hope this helps. Also, I've got copies of the TNIK manuscript figures that you need. Would you like me to fax them to you?

Nicole

-----Original Message-----

From: Ying Luo [mailto:yluo@rigel.com]
Sent: Sunday, August 29, 1999 2:44 PM
To: Nicole Verona
Subject: Re: FW: info

2868 PAN is from PCNA screening. tankyraseH is from CIP screening. CIP is also called p21. R0101 has an entry in GenBank with full length sequence with a name called KIAA0101. No functional annotation about R0101. PP5 was cloned and published before. The novelty is we can link PP5 to RAD9, a cell cycle checkpoint control protein. You should have all figures of TNIK manuscript already. TNIK nucleotide sequences are attached. PAN nucleotide sequence is already in Genbank.

Ying

At 03:21 PM 8/26/99 -0700, you wrote:

>Hi Ying!

>

>Here are some of the questions I need to discuss with you.

>

>Nicole

>

>-----Original Message-----

>From: Dolly Vance [mailto:dvance@flehr-iplaw.com]

>Sent: Friday, August 20, 1999 1:42 PM

>To: nverona@rigel.com

>Subject: info

>

>

>Dear Nicole,

>Hope you're well. Here's a complete list of what I am missing from the

>initial 9 disclosures.

>

>1) The names of binding partners (if any actual) for CAH and

>tankyraseH.

>2) The nucleic acid and amino acid sequences for PAN and TNIK

>(actually, all figures that go with the manuscript for TNIK).

>3) Please confirm that R0101 and PP5 are NOT novel, and that all

>others are novel.

>

>Thanks. Dolly

>P.S. I understand your hours are reduced. Any chance you can give me a

>time frame for providing the above information? Thanks again, Dolly

>

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RIGEL, INC.

FLEHR, HOHBACH, TEST,
ALBRITTON & HERBERT

1999 OCT -1 AM 10:09

RECEIVED

Exhibit H 09/843 149

September 30, 1999

Ms. Dolly Vance
Flehr, Hohbach, Test, Albritton and Herbert LLP
4 Embarcadero Center, Suite 3400
San Francisco, California 94111-4187

Dear Dolly,

Enclosed are documents pertaining to the cell-cycle patent applications that you requested.

The documents include:

1. TankyraseH abstracts involving TRF, P21, and PARP
2. TankyraseH nucleotide sequence alignment report
3. TankyraseH amino acid sequence alignment report
4. R0101 figures with corrected CDK 2, 3, and 4 labels
5. Mkinase nucleotide and amino acid sequences with its kinase domain and nuclear localization sequence (NLS) highlighted

Additional information will be sent to you next week.

Please call or email me if you have any questions.

Sincerely,

Nicole Verona

Nicole Verona